Exhibit 5

EGG SCIENCE AND TECHNOLOGY

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CHAPTER 13

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Desugarization

INTRODUCTION

Dehydrated egg prepared from native liquid egg is subject to undesirable changes during storage; these include loss of solubility, decreased functionality, and the formation of off-color and objectionable flavor.

The United States dried-egg industry began an era of expansion in the early 1930's. During this period, imports from China gradually decreased as a result of the Chinese Civil War, high import duties on frozen and dried egg, and low shell egg prices in the United States. Because of previous dependency upon the Chinese products and the success of the Chinese in keeping their methods secret, the domestic producers of dried albumen lacked a fundamental knowledge of their product. They were unable to produce dried albumen comparable to the Chinese product in storage stability and functionality.

American scientists and dried-egg producers knew that the Chinese albumen underwent a fermentation process prior to drying. However, it was generally believed that fermentation was necessary only for "thinning" the thick albumen to render it suitable for drying and to improve the solubility and whipping qualities of the powder. Not surprisingly, the uncontrolled fermentation of liquid egg by organisms which just happened to be present was considered objectionable by some individuals. However, attempts to produce acceptable dehydrated egg products without a fermentation process were not successful. An excellent historical review covering the early development of the United States egg-products industry was prepared by Koudele and Heinsohn (1960).

World War II created a strong demand for dried-egg products, particularly powdered whole egg, for use by the armed forces. The need for an egg product which remained palatable under severe conditions of storage and transportation stimulated research into the physical, chemical and biological changes associated with the dehydration of egg products. The results of numerous studies elucidated the role of glucose in the storage stability of dried egg products.

REACTIVITY OF GLUCOSE IN DRIED EGGS

Much research has been conducted on the interaction of glucose with other egg components which result in deterioration in the quality of dried-egg products. Two major reactions have been defined: the glucose-protein (Maillard) reaction and the glucose-cephalin reaction.

Interaction of Glucose and Proteins

The condensation of sugars with amino acids was first studied by Maillard in 1912, thus the reaction bears his name. The initial bonding between the glu-

cuidic hydroxyl groups of sugars and the amine groups of peptides and proteins is followed by other changes which result in the formation of brownish-colored products. Because of these end products, the overall process is often called the "browning" reaction.

Unfermented egg white powder darkens when heated to high temperatures for a substantial length of time. While conducting studies on the "thinning" of egg white by added trypsin, researchers noted that, although the enzyme liquefied the albumen, the subsequent dried powder developed off-colors during storage. It was suggested that this color change was due to a combination of the egg sugar with aminonitrogen. As further evidence of the importance of glucose in dried albumen, fermentation of egg white converts the glucose present to acid, and this process ensures solubility and color stability when the powder is held in storage. The reaction between glucose and the amino groups of proteins is responsible, at least in part, for both discoloration in dried eggs and fluorescence in salt extracts. Glucose is reactive with several of the egg proteins.

A sequence of reactions was postulated to explain the various deteriorative changes occurring in dried egg albumen. It was suggested that the initial reaction between glucose and the egg proteins is followed by additional reactions, one resulting in fluorescence and color alterations and another in insolubility. The former reaction was believed to proceed at a faster rate than the latter. Additional evidence substantiated the theory that the Maillard reaction was responsible for the changes in dried albumen.

Interaction of Glucose and Cephalin

Various researchers have presented evidence that some deteriorative changes occurring in dried whole egg and yolk are independent of the glucose-protein reaction. An ether-soluble brown substance was extracted from darkened whole egg powder. Chemical analysis of the substance indicated derivation from a phospholipid, specifically cephalin. The results of further testing suggested that the discoloration of the dried whole egg was due, at least in part, to a reaction between a cephalin amino group and aldehydes. Major changes resulting in loss of palatability take place in the fatty constituents of the egg. Ether extracts were more reliable than salt water extracts for the determination of fluorescence values as indices of palatability.

Glucose is the reactive aldehyde involved in the cephalin amine-aldehyde reaction. The changes which occurred in the phospholipid fraction of stored whole egg powder were essentially eliminated by the removal of glucose from the liquid before drying. In a study on the relative influence of the glucose-protein and glucose-cephalin reactions in whole egg powder, it was shown that loss in baking quality is associated chiefly with the glucose-protein reaction, while the glucose-cephalin reaction is involved in off-flavor development.

METHODS OF DESUGARIZATION

The factors which influence the quality and storage stability of dried eggs have been studied extensively. Moisture level, storage temperature, particle size, acidity, carbohydrate addition, and gas packing have been considered individually and in combination as to their influence on the stability of dried eggs. While each of these factors can be utilized in some beneficial manner, none has proven as successful in producing a stable egg product as the removal of glucose from the liquid prior to drying.

Spontaneous Microbial Fermentation

The fermentation of liquid eggs by contaminating microorganisms was practiced by the egg-drying industry until about the mid-1940's. The natural fermentation of albumen at 23.9–29.4°C can be described as follows:

The whites usually remain quiescent for several days (the exact time depending on the amount of bacterial contamination), during which time there is some increase in the amount of thin, watery white at the expense of the thick, jelly-like white. Gradually an acid fermentation with a mild, characteristic odor sets in and the thick white begins to gather at the surface. The mucin fibers in this thick white contract under the influence of the acid produced. Usually after six to seven days they are practically free of thin white and collect as a layer of very stringy and cloudy material on the surface of the fermenting albumen. A considerable amount of the carbon dioxide present in the egg white is liberated as the acid increases, but the evolution usually stops about the sixth or seventh day of fermentation. The body of the thin white is at first very stringy, but after the fermentation is complete, it becomes watery and drips freely from the end of a glass rod. After this period the egg white has reached a stage when it is usually considered to be ready for drying. If the albumen is allowed to continue fermenting, there is a loss of acid (increase in pH), and usually very objectionable odors begin to develop.

Early bacteriological tests on samples of commercially fermenting egg white revealed a predominance of *Aerobacter aerogenes* or *Escherichia freundii* (these organisms are classified now in the genera *Enterobacter and Citrobacter*, respectively) with few other contaminants. Egg white fermented by either of these organisms yielded a bright, crystalline, granular product on drying. Fermentation by proteolytic bacteria such as *Proteus*, *Serratia* or *Pseudomonas* resulted in an inferior product.

While natural fermentation did make possible the production of egg powders with adequate storage stability, it often caused problems in the freshly dried material. Not the least of these problems was the potential health hazard resulting from the growth of pathogenic bacteria, such as *Salmonella*, in the fermenting liquid. The dehydration process, as commonly practiced in the production of powder of 4 to 6% moisture content, was not highly destructive to *Salmonella* organisms.

Controlled Bacterial Fermentation

A patent issued in 1931 described a process whereby liquid egg white was inoculated with an acid-producing organism such as "lactic acid bacillus." The following were claimed as advantages of their process over spontaneous fermentation: (1) reduction of the time required from 48 to 60 hours or more to 24 hours or less; (2) production of a more uniform powder; (3) less hazard from pathogens; and (4) reduced possibility of the production of putrefactive odors.

An acceptable dried albumen was obtained from liquid which had been fermented by pure cultures of coliform organisms.

Stabilization of egg white was achieved by inoculating the liquid with a culture of *Streptococcus*, although some flavor changes resulted from the fermentation process. Also, desugarization of liquid egg yolk by *Pseudomonas* was not highly beneficial to storage stability and resulted in the formation of off-flavor.

Species of *Streptococcus* and *Lactobacillus* were utilized to desugar whole egg. They found that these organisms could eliminate practically all the sugar from whole egg within 24 hours, but that a similar amount of sugar in inoculated egg white was decreased very little. Evidently, *Streptococcus lactis* does not successfully remove glucose from egg white unless large numbers of the organism are utilized or yeast extract is added to the cells as a growth promoter prior to inoculation. *Enterobacter aerogenes* did multiply in egg white and simultaneously converted the egg sugar to acid.

More recently, Galluzzo *et al.* (1974) were able to simultaneously desugar whole egg and impart flavors and/or aromas characteristic of dairy products by fermentation with *Strep. diacetilactis*. In order to obtain best growth and diacetyl production, it was necessary to heat the whole egg for 20 min. at 65°C and adjust the pH to 5.5 with citric acid.

A 1% by weight of resting cells of *Streptococcus lactis* was used to desugar egg white. The glucose level was reduced from 0.320% to 0.006% in 1.5 hours at 37°C. The high numbers of streptococci (5×10^8 per milliliter of egg white) and short fermentation time prevented multiplication of Gram-negative organisms. Angel food cakes prepared from pan-dried albumen fermented by concentrated streptococci were of good quality.

Much of the work conducted toward development of cultures for the bacterial fermentation of egg albumen has been carried out by individual companies of the egg industry. The results of these studies, and the cultures evolved, are generally regarded as confidential.

Yeast Fermentation

The use of pure cultures of yeast to remove glucose from liquid egg was introduced in the mid-1940's. Albumen and whole egg were desugared with *Saccharomyces apiculatus*. The glucose level of the albumen was reduced from

0.5 to 0.05% after 3 hr incubation at 37°C. However, the high level of yeast cells (1%) added to the liquid white resulted in a powder with an objectionable yeasty flavor. In another study, a relatively small inocula of *Saccharomyces cerevisiae* was used to remove the glucose from egg white. The glucose conversion was enhanced greatly by the presence of 0.1% yeast extract. The acid produced was not sufficient to cause precipitation of the mucin.

The development of yeast fermentation by *Saccharomyces cerevisiae* is credited to studies carried on in the laboratories of Armour and Company, Chicago, Illinois. Fermentation of whole egg with 0.2–0.4% by weight of moist baker's yeast at 22–23°C depleted the sugar within 2 to 4 hours. The final product was substantially free of yeast flavor. Later, the flavor of yeast-fermented whole egg was improved by centrifuging the sugar-free liquid to remove the yeast cells.

Strains of *Saccharomyces* produce a more palatable whole egg powder than strains of *Torulopsis*. Acidification of the whole egg melange to a pH below 6.0 increased the rate of fermentation, the optimum temperature of incubation being 30°C. A spray-dried whole egg with no significant yeast flavor was produced from liquid fermented with 0.07 to 0.15% dry weight of yeast in 2 to 3 hr.

Yeast fermentation is a practical means of removing the glucose from whole egg. An occasional development of mustiness in yeast-fermented whole egg powder during storage is noted. Studies show that consumers have strong preference for angel cakes made with frozen egg whites over angel cakes prepared with yeast-fermented, pan-dried albumen. However, the yeast-fermented angel cakes did not contain vanilla flavoring. When this ingredient was incorporated into the formulation, the differences between the two samples of cakes were not significant.

The advantages of yeast fermentation have been summarized as follows:

(1) It causes little change in acidity and may thus eliminate the need for neutralization and minimizes loss of mucin.
(2) The growth of contaminating organisms is reduced because of the short incubation time required by the process.
(3) Moist yeast is readily available in a convenient form.
(4) Fermentation without artificial means of aeration favors conversion of the sugar rather than multiplication of yeast cells.
(5) The process does not cause development of objectionable odors or flavors or introduce undesirable by-products.

Enzyme Fermentation

Glucose oxidase was found in cultures of *Aspergillus niger* and *Penicillium glaucum* by Müller in 1928. He showed that in the presence of molecular oxygen the enzyme catalyzed the oxidation of glucose to gluconic acid. Franke and Lorenz in 1937 further characterized the reaction by finding that hydrogen

peroxide was also a product of the enzyme activity. By using radioisotopes, Bentley and Neuberger later showed that the oxygen atoms of the hydrogen peroxide produced by the reaction were derived from molecular oxygen, and that the enzyme catalyzes the transfer of hydrogen from glucose to oxygen. Thus glucose oxidase is, in actuality, an oxidoreductase.

An enzyme system comprised of glucose oxidase and catalase, an enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen, was developed for use in deoxygenation of foods and beverages. The activity of the glucose oxidase-catalase system in equation form is:

$$C_6H_{12}O_6 + O_2 + H_2O \xrightarrow[\text{oxidase}]{\text{glucose}} C_6H_{12}O_7 + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Net reaction

$$\underset{\text{glucose}}{2C_6H_{12}O_6} + O_2 \xrightarrow[\text{system}]{\text{enzyme}} \underset{\substack{\text{gluconic}\\ \text{acid}}}{2C_6H_{12}O_7}$$

From the net reaction it is apparent that the enzyme system can be used to stabilize foods by (1) the removal of oxygen in the presence of excess glucose, and (2) the removal of glucose in the presence of readily available oxygen. For the latter usage, it is convenient to supply excess oxygen by the addition of hydrogen peroxide to the liquid being desugared.

The first report of a comprehensive study on the enzymatic desugaring of an egg product was that of Baldwin *et al.* in 1950. They used a commercial preparation of glucose oxidase-catalase to remove the glucose from large volumes of albumen. They found the process easy to control, reproducible and capable of yielding a uniform powder with no objectionable odors traceable to the desugaring process. A few months later, other researchers reported that powder made from enzyme-treated albumen had no off-odors or flavors and produced acceptable angel cakes.

Empirical relationships were developed between glucose level, time, enzyme level and hydrogen peroxide demand to utilize the enzyme method for desugaring albumen more efficiently.

Enzyme treatment of egg yolk was demonstrated to improve the storage stability of the dry solids at 35°C, as determined in doughnut mixes. The untreated control solids yielded doughnuts of lower fat content and decreased palatability. Similar, but less dramatic results have been obtained with yolk solids held at 20°C.

CURRENT INDUSTRY GLUCOSE REMOVAL PRACTICES

A detailed description of glucose removal practices used currently in the egg industry is difficult because of the confidential nature of this area of processing.

A few generalizations may be made, however, concerning the overall procedures employed in desugarization.

Egg White

The removal of glucose from egg white is done almost entirely by the controlled bacterial fermentation process. While both the glucose oxidase enzyme and yeast methods may be used, the bacterial culture method has both functional and monetary advantages. The use of bacterial fermentation results in a high whipping egg white solids product with good flavor and solubility at a nominal cost in terms of labor and materials. However, bacterial fermentation of yolk-containing egg is considered an unsatisfactory method because it yields products with off-flavors and odors.

In some companies the bacteria culture, or "bug," is well identified, while in others it is characterized by its ability to convert the glucose effectively rather than by specific genera and/or species.

It is common practice for companies using the bacterial fermentation process to initiate growth of the culture in a small batch of albumen. The albumen is first pasteurized and then acidified to pH 7.0 to 7.5 with food grade citric or lactic acid. The liquid is then inoculated with the proper culture and held at 30 to 33°C. Extreme caution is taken to prevent contamination of the albumen by foreign bacteria, yeast or mold. Additional transfers of the actively fermenting culture to pasteurized acidified egg white can be made to increase the quantity of culture. The fermentation is stopped short of complete sugar depletion by reducing the temperature while the bacteria are in a high state of metabolic activity. The culture is then frozen in small quantities for use as inocula in the fermentation of large batches of egg white. This stepwise preparation of inocula allows time for bacteriological analyses of the culture to ensure the absence of undesirable organisms.

The bacterial fermentation process for large batches of egg white is essentially the same as that used to produce small amounts of inocula, except that the glucose removal is allowed to go to completion.

Whole Egg and Yolk

The glucose oxidase-catalase enzyme system is used almost exclusively on whole egg and other yolk-containing egg products. This procedure may be carried out at an elevated temperature of 30 to 33°C, or at a low temperature, 10°C. The latter temperature requires a longer fermentation time but is a deterrent to the growth of undesirable microorganisms during the processing period. The enzyme method may be employed in any practical size batch of whole egg or yolk. Adjustment of pH (required in egg white) is not necessary in yolk which is naturally close to the optimum glucose oxidase reaction pH of 6.0. However, pH adjustment with citric or lactic acid may be required in some whole egg. Since the level of enzyme added is determined by the rate of reaction desired, tem-

perature of egg, strength of enzyme purchased, and the amount of native glucose to be removed (yolk has a higher glucose content than whole egg), it is impossible to specify the exact amount of enzyme required for the process. Proper use levels are best obtained from suppliers and personal experience.

When pH, temperature, and enzyme are properly adjusted, hydrogen peroxide is metered into the product at a level determined by the amount of enzyme and type of egg product. Considerable caution is required in adding hydrogen peroxide to the egg because of foaming from evolved oxygen. Normally, the rate of peroxide to the egg because of foaming from evolved oxygen. Normally, the rate of peroxide addition is reduced during the latter part of the fermentation as the amount of glucose in the egg is decreased.

The use of yeast in glucose removal, while applicable to all liquid egg, is employed primarily in the desugaring of yolk-containing products. This method is probably the simplest of all to perform. Commercially, common baker's yeast is employed at a level of 3.4 lb per 1000 lb of egg. Both the amount of yeast and the temperature are controlling factors in the rate of glucose removal. Temperatures of 30°C are considered optimum for this process. If carefully controlled, the yeast fermentation process results in no multiplication of yeast cells during fermentation (resting fermentation) with little or no yeast flavor in the finished product. The yeast procedure removes not only the glucose present but other trace reducing sugars which are not removed by the glucose oxidase method (an interesting sidelight, but of little practical importance).

The fermentation process is deemed complete when glucose is not detected by Somogyi sugar reagent or Clinistix®. A quick test which is particularly suited for albumen involves heating 1 to 2 ml of fermented liquid on a glass Petri dish with an infrared lamp for 10 to 15 minutes. The absence of browning shows that insufficient glucose is present to give a reaction.

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